

AMENDMENTS TO THE SPECIFICATION:

Please replace original paragraph [0023] with the following amended paragraph.

[0023] Figure 7. A: The alignment of the gram-positive promoter consensus with the sequence determined from "PCR walk 6-9" of the GP1223 insert.

B. A sequence (SEQ ID NOS: 48-49) containing dyad symmetry followed by a stretch of thymidine residues, approximately 150 nucleotides upstream of the -35 region, that conforms to a prokaryotic factor-independent RNA polymerase terminator sequence.

Please replace paragraph [0037] with the following amended paragraph.

[0037] TABLE 1. Bacterial strains, plasmids and oligonucleotides

Strain, plasmid or oligo	Relevant markers and characteristics	SEQ ID NO.	Reference or source
Strains			
<i>E. coli</i>			
INVαF	F <i>endA1 recA1 hsdR17</i> (r _k ⁻ , m _k ⁺) <i>supE44 thi-1 gyrA96 relA1</i> Ö80 <i>lacZ</i> ÄM15 Ä(<i>lacZYA-argF</i>)U169		Invitrogen
XL1 Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F <i>proAB lacI</i> ªZÄM15 Tn10 (Tet ^r)] ^c		Stratagene
<i>S. gordonii</i>			
V288	Wild Type (ATCC 35105)		ATCC
GP1223	M protein recombinant strain that expresses M6 protein (<i>S. pyogenes</i>) residues 1 to 16 fused to residues 222-441 and contains an <i>aphIII</i> gene, Km ^r		G. Pozzi
SP-02	M protein recombinant strain, p635:M/ <i>aphIII</i> in V288, Km ^r		This work
SP-04	M protein recombinant strain, pLacG:M/ <i>aphIII</i> in V288, Km ^r		This work
635/ermC	M protein recombinant strain, p635/ermC in V288, Em ^r		This work
LacG/ermC	M protein recombinant strain, pLacG/ermC in V288, Em ^r		This work
Plasmids			
pCR2.1	Km ^r , Amp ^r		Invitrogen
pCR2.1:635	1.1-kb PCR-amplified 6-35 walk from V288 cloned into pCR2.1 at EcoRI, Amp ^r		This work
p635(NdeI)	NdeI site incorporated in between orf 1 and orf 2 in pCR2.1:635, Amp ^r		This work

Strain, plasmid or oligo	Relevant markers and characteristics	<u>SEQ ID</u> <u>NO.</u>	Reference or source
pCR2.1:6-86	1733 bp PCR amplified 6-86 walk containing most of the <i>lacG</i> gene and part of the <i>lacE</i> gene from V288 cloned into pCR2.1 at EcoRI, Amp ^r		This work
pLacG	Derivative of pCR2.1 carrying 1.7-kb <i>lacE/G</i> cassette with NdeI incorporated within the <i>lacG</i> ORF, Amp ^r		This work
p635(NdeI) derivatives			This work
p635/ermC	1.2-kb <i>ermC</i> fragment from pSMB104 cloned into NdeI site, Amp ^r		This work
p635:M/aphIII	2.7-kb M/ <i>aphIII</i> fragment from GP1223 cloned into NdeI site, Amp ^r		This work
pLacG derivatives			
pLacG/ermC	1.2-kb <i>ermC</i> fragment from pSMB104 cloned into NdeI site, Amp ^r		This work
pLacG:M/aphIII	2.7-kb M/ <i>aphIII</i> fragment from GP1223 cloned into NdeI site, Amp ^r		This work
Oligonucleotides			
CF4	5'-AATAGGGCTCGAGCGGC- 3'	<u>1</u>	23
CF5	5'-GGATCCTAATACGACTCACTATAGGGC- 3'	<u>2</u>	23
CF6	5'-AATAGGGCTCGAGCGGC- 3'	<u>1</u>	23
CF7	5'-ACCTGCCC-(c3-Icaa-CPG spacer)	<u>3</u>	3
CF35	5'-CGATTGACATAGAAATAAATTGGAG- 3'	<u>4</u>	3
CF43	5'-GTTTGGTGACCTATAGTCAGTG- 3'	<u>5</u>	3
CF45	5'-TGGATGGCATGAATGTATAGAT- 3'	<u>6</u>	3
TB59	5'-AAAGAAGCATAACATATGTCAAAACAAG-3'	<u>7</u>	This work
TB85	5'-ACACTTCATCACTTTGATACCCCAGA- 3	<u>8</u>	This work
TB86	5'-CCATTTGACCATGAGAAGACATCCATC-3'	<u>9</u>	This work
TB95	5'-AAATCTCCATTTGAATGAAGTGCCTCTGGGG-3'	<u>10</u>	This work
TB96	5'-GTCCACAAAGTGCTCAATATTATCCCGATTGAG-3'	<u>11</u>	This work
TB100	5'-AGGGCGTCAGAGAATCTCCAACCCATATACC- 3'	<u>12</u>	This work
TB103	5'-GGAATTCCATATGCGGATAATAAATATATATAAACG-3'	<u>13</u>	This work
TB104	5'-GGAATTCCATATGCGATTACAAAAAATAGGCACACG-3'	<u>14</u>	This work
TB107	5'-GCAGGAGTGGACGAAGAAGCTCC-3'	<u>15</u>	This work
TB117	5'-GGATCCCATATGTAAGGAGCATAAAAATGGC-3'		This work

Please replace paragraph [0054] with the following amended paragraph

[0054] **TABLE 2. Bacterial strains, plasmids, and oligonucleotides**

Strain, plasmid, or oligonucleotide	Relevant markers and characteristics	Reference or source
Strains		
<i>E. coli</i>		
INVaF'	F' <i>endA1 recA1 hsdR17(r_K, m_K⁺) supE44 thi-1 gyrA96 relA1ö80lacZÄM15 Ä(lacZYA-argF)U169</i>	Invitrogen
<i>S. gordonii</i> Challis		
V288	Wild-type (ATCC 35105)	ATCC
GP204	Spontaneous Sm ^r mutant of V288	Pozzi et al., 1988
GP230	Recombinant strain contains the <i>emm6</i> gene (<i>S. pyogenes</i>) and an <i>ermC</i> gene, Em ^r , parent strain (V288)	Pozzi et al., 1992
GP251	Recombinant recipient strain contains the <i>cat</i> gene flanked by 145 bp of <i>emm6</i> gene and 202 bp of <i>ermC</i> gene, Cm ^r , parent strain (GP230)	Oggioni et al., 1996
GP1214	Recombinant strain that expresses M6 protein (<i>S. pyogenes</i>) residues 1 to 16 fused to residues 222-441 and contains an <i>ermC</i> gene, Em ^r , parent strain (GP251)	Oggioni et al, 1994
GP1218	Recombinant strain that expresses M6 protein (<i>S. pyogenes</i>) residues 1 to 16 fused to residues 222-441 and contains an <i>aphIII</i> gene, Km ^r , parent strain (GP1214)	Oggioni et al, 1994
GP1223	Recombinant strain that expresses M6 protein (<i>S. pyogenes</i>) residues 1 to 16 fused to residues 222-441 and contains an <i>aphIII</i> gene, Km ^r , and has been converted to Sm ^r , parent strain (GP1218)	Oggioni et al, 1994

Strain, plasmid, or oligonucleotide	Relevant markers and characteristics	Reference or source
Plasmids		
pCR2.1	Km ^r , Amp ^r	Invitrogen
pSMB104	Contains the sequences encoding the CRR of M6 protein (<i>S. pyogenes</i>) residues 1 to 16 fused to residues 222-441 in tandem with an MspI/ClaI fragment of pE194 () encoding ermC cloned into pBluescript SK-.	Oggioni et al, 1994
Oligonucleotides		
CF4 (SEQ ID NO: 16)	5'-CTAATACGACTCACTATAGGGCTCGAGCG GCCGCCC GGGCAGGT-3'; Adaptor	Siebert et al, 1995
CF5 (SEQ ID NO: 2)	5'-GGATCCTAATACGACTCACTATAGGGC-3'; AP1	Siebert et al, 1995
CF6 (SEQ ID NO: 1)	5'-AATAGGGCTCGAGCGGC-3'; AP2, SEQ	Siebert et al, 1995
CF7	5'-ACCTGCCC-(C3-Icaa-CPG spacer); AP1	This study
CF8 (SEQ ID NO: 17)	5'-TCTAGAGGTACCTTCTCGTGCTTTGTCCGG -3'; PCR (GP1223)	This study
CF9 (SEQ ID NO: 18)	5'-TACCGTCCCCCTAGGAAACACTCTTGAC-3'; SEQ, PCR (GP1223)	This study
CF10 (SEQ ID NO: 19)	5'-TGACTTACTGGGGATCAAGCCTGATTGGG AG-3'; PCR (GP1223)	This study
CF11 (SEQ ID NO: 20)	5'-AAGTACATCCGCAACTGTCCATACTCTGAT G-3'; PCR (GP1223)	This study
CF14 (SEQ ID NO: 21)	5'-GTTTTTCGTGTGCCTATTTTTTGTG-3', SEQ 1223	This study

Strain, plasmid, or oligonucleotide	Relevant markers and characteristics	Reference or source
CF15 (SEQ ID NO: 22)	5'-GAGCGCATCGAAAATGCTGTTG-3'; SEQ, PCR (GP204)	This study
CF16 (SEQ ID NO: 23)	5'-CTCAGTGTAAGAGGAAATCC-3'; SEQ	This study
CF17 (SEQ ID NO: 24)	5'-GAGTTTCAATGGTCTTGTCTGG-3'; SEQ, PCR (GP204, GP1223)	This study
CF18 (SEQ ID NO: 25)	5'-CTTGAAAAGCCTGAGGGCTGGTTAC-3'; SEQ, PCR (GP204)	This study
CF19 (SEQ ID NO: 26)	5'-CTTGACCTTTGGTACCTTTGAC-3'; SEQ	This study
CF20 (SEQ ID NO: 27)	5'-GATAGTCACACGGCTACTCACG-3'; SEQ	This study
CF21 (SEQ ID NO: 28)	5'-CGTGAGTAGCCGTGTGACTATC-3'; SEQ	This study
CF22 (SEQ ID NO: 29)	5'-GTCCATAGAGTTTGGATCCAAG-3'; SEQ	This study
CF23 (SEQ ID NO: 30)	5'-GTCAAAGGTACCAAAGGTCAAG-3'; SEQ	This study
CF24 (SEQ ID NO: 31)	5'-CCAGAAATTCGCGATATGAAC-3'; SEQ	This study
CF25 (SEQ ID NO: 32)	5'-GAATGAATCCAGATAAGGTGC-3'; SEQ	This study
CF26 (SEQ ID NO: 33)	5'-GATATCTTCAACTCATGGGATTAC-3'; SEQ, PCR (GP204)	This study
CF27 (SEQ ID NO: 34)	5'-CAAGATTCTCACCAGTTTTATG-3'; SEQ	This study
CF28 (SEQ ID NO: 35)	5'-GCTGCGATGCTTATGATTACC-3'; SEQ	This study
CF29 (SEQ ID NO: 36)	5'-GCTACCAATGCTGACAATAG-3'; SEQ	This study

Strain, plasmid, or oligonucleotide	Relevant markers and characteristics	Reference or source
CF31 (SEQ ID NO: 37)	5'-CCTAAGCAGTTTCTCAAGTTG-3'; SEQ	This study
CF32 (SEQ ID NO: 38)	5'-CATGTTGCCTATCGTCCAGC-3'; SEQ PCR (GP204, GP1223)	This study
CF35 (SEQ ID NO: 3)	5'-CGATTGACATAGAAATAAATTGGAG-3'; SEQ, PCR (GP204)	This study
CF36 (SEQ ID NO: 39)	5'-CTATAGTCAGTGTGGTTTAGACAAGC-3'; SEQ	This study
CF39 (SEQ ID NO: 40)	5'-GATTATGCTGAATCAAATAGTC-3', SEQ	This study
CF40 (SEQ ID NO: 41)	5'-GAGCACGATAGTAGTCAATCAC-3'; SEQ	This study
CF41 (SEQ ID NO: 42)	5'-CAATTTTGGACTGATACGATGGC-3'; SEQ	This study
CF42 (SEQ ID NO: 43)	5'-CTGTTCTTCCAACCTTTTTCAGC-3'; SEQ	This study
CF43 (SEQ ID NO: 4)	5'-GTTTGGTGACCTATAGTCAGTG-3'; SEQ	This study
CF44 (SEQ ID NO: 44)	5'-ATCTATACATTCATGCCATCCA-3'; SEQ	This study
CF45 (SEQ ID NO: 5)	5'-TGGATGGCATGAATGTATAGAT-3'; SEQ	This study

Please replace paragraph [0062] with the following amended paragraph.

[0062] **The region upstream of the GP1223 insert contains regulatory signals.** Immediately upstream of the GP1223 insert, sequences which conform to the consensus for promoters from gram-positive organisms (DeVos, W.M. 1987 FEMS Microbiol. Rev. 46:281-295; Graves, M.C., and J.C. Rabinowitz. 1986. J. Biol. Chem. 261:11409-11415) were found. The alignment of the gram-positive promoter

consensus with the sequence determined from "PCR walk 6-9" is shown in Figure 7A. This sequence shows the following features in common with the gram-positive promoter consensus: (i) the canonical -35 and -10 sequences; (ii) a spacing between those hexanucleotides of 16 to 18 nucleotides; (iii) the conserved dinucleotide sequence TG, immediately preceding the -10 sequence; and (iv) the AT-rich regions upstream of the -35 sequence (AT-box). Approximately 150 nucleotides upstream of the -35 region, a sequence containing dyad symmetry followed by a stretch of thymidine residues conforms to a prokaryotic factor-independent RNA polymerase terminator sequence (Figure 7B). Also, a region containing five direct repeats, 4 perfect and 1 imperfect, of 18 nucleotides (AGTTTAAAATCTTTATTC)(SEQ ID NO: 4) was observed between the terminator and the promoter sequences (Figure 7B). Upstream of the terminator sequence, the nucleotide sequence of the 881-bp "PCR walk 6-9" also contained a partial ORF (designated ORF2, see Figure 6) encoding 169 residues with no apparent functional homologies in the databases at present. The sequence of ORF 2 had not terminated when the walk fragment ended at an EcoRV site to which the walking adaptor was ligated.